

Processing *Vernonia amygdalina* Delile to Reduce Competition for Food Between Humans and Livestock

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Abstract

This study evaluated and endorsed optimal processing methods for *Vernonia amygdalina* Delile with the dual objective of producing a palatable, de-bittered vegetable (*ndole*) for human consumption and a nutrient-rich co-product (*Vernonia amygdalina* Wash-water-VaWW) for livestock feed. What distinguishes this study is its comprehensive, side-by-side evaluation of both edible and co-product streams from 21 debittering protocols, a level of comparative detail not presented in prior research. Nutritional values and antioxidant trade-offs are quantified for the first time across washing, abrasion, boiling, and potash-based methods. Twenty-one traditional processing methods from existing literature were first standardized under laboratory conditions and then scaled up to household conditions. Six most suitable methods—selected for their de-bittering effectiveness and efficiency—were subsequently evaluated for their impact on nutrient and antioxidant retention in both the final product and the wash-water. Results revealed a significant trade-off between processing time and nutrient retention. While Method 21 (heating with potash and water) was significantly ($P < 0.05$) the most time-efficient, requiring just 5 minutes to process 1000g of leaves, it resulted in significant ($P < 0.05$) nutrient and antioxidant loss in the final product. Conversely, Method 4 (ordinary abrasion until foaming stops) was found to be the superior ($P < 0.05$) processing method for nutrient retention. Method 4 consistently resulted in the highest retention of protein (content statistically similar ($P > 0.05$) to unprocessed leaves), Vitamin C, and minerals in the de-bittered *ndole*. Boiling methods (Methods 9 and 14) led to a greater loss ($P < 0.05$) of nutrients in the *ndole* but, consequently, produced VaWW with a higher nutrient content, confirming the transfer of these compounds from the leaves to the VaWW. In conclusion, this research identifies a superior processing method that maximizes nutrient retention for human food while simultaneously generating a valuable co-product for livestock. The utilization of *Vernonia amygdalina* wash-water as an animal feed additive provides a sustainable strategy to mitigate competition for this valuable food source. Policy adoption of abrasion-based protocols is recommended for communities prioritizing nutritional retention. Wash-water co-products should be promoted as sustainable livestock supplements, reducing feed costs and food competition.

Keywords: *Vernonia amygdalina*, food security, livestock feed, nutrient retention, co-product, processing method

1. Introduction

The increasing global population and rising demand for animal protein have intensified the competition for food resources between humans and livestock (Makkar, 2018). A significant portion of the global animal feed supply, particularly for monogastric animals like pigs and poultry, consists of crops that are also consumed by humans, such as cereals. A key strategy to mitigate this competition is to integrate livestock into a circular food system by upcycling agricultural and food processing by-products into valuable feed sources. Examples include corn gluten feed from ethanol production or whey from cheese-making (Naderi et al., 2022). In recent years, a new challenge has emerged as various spices and vegetables, traditionally consumed by humans, are increasingly being used as natural and sustainable alternatives to synthetic growth promoters in animal feeds. These include garlic (*Allium sativum*) (Nghonjuyi et al., 2023), ginger (*Zingiber officinale*) (Eltazi, 2014), thyme (*Thymus vulgaris*) (Ezzat et

al., 2020), and bitterleaf (*Vernonia amygdalina*) (Tokofai et al., 2023). This trend highlights the need to develop strategies that minimize the direct competition for these valuable food resources. Therefore, this research focuses on the development of *Vernonia amygdalina* Wash-water (VaWW) as a co-product of bitterleaf processing, transforming it into a viable feed resource for farm animals.

Vernonia amygdalina, a perennial African leafy vegetable widely distributed in Africa, Asia, and Brazil, is considered a functional food due to its rich content of nutrients and medicinal compounds that combat hidden hunger (Degu et al., 2024; Uchegbu et al., 2022). It goes by different local names in different cultures—'ndole' in Cameroon, 'awonwono' in Ghana, 'onugbu' in Nigeria or 'aloman' in Benin (Houegban et al., 2024). In many places, it grows in the wild, but it is also cultivated in farms and homesteads and ranks among the most commercialised vegetables (Houegban et al., 2024). It is commonly called "bitterleaf" due to its characteristic bitter taste, primarily attributed to its content of phytochemical compounds including saponins, tannins, glycosides, alkaloids, and the sesquiterpene lactones: vernodalol, vernomygdin, vernolide, hydroxyvernolide, and vernodalol (Mitsuo et al., 1993; Luo et al., 2011; Tura et al., 2024; Ampem Danso et al., 2024). The bitter tastes compel it to be commonly prepared for human consumption by washing the leaves (Tekou et al., 2018). The wash-water, a by-product of this process, is typically discarded as waste. However, this wash-water contains a substantial amount of nutrients and other biologically active compounds, which could serve as a valuable resource, for example, as an organic fertilizer (Fabunmi, 2019) or as a growth promoter for farm animals. By utilizing the wash-water in animal nutrition, its use would not compete with human consumption of the de-bittered leaves, which are locally known as "ndole" in Cameroon.

While various methods for processing *Vernonia amygdalina* for human consumption have been documented (Kelechi et al., 2015), a significant gap exists in the literature regarding a comparative evaluation of the co-products (ndole and VaWW) generated by these different methods. Some processing methods utilized and reported include blanching, squeezing with salt; abrasion with palm oil, heating with potash, ordinary abrasion, soaking, squeeze wash and sun-drying (Adeboye & Babajide, 2007; Yakubu et al., 2012; Abioye et al., 2014; Kelechi et al., 2015; Edeani, 2021). Each of the utilized and reported methods produces VaWW, in various quantities over different time spans. It remains unclear which processing technique simultaneously optimizes the quality of the de-bittered product for human consumption and the nutritional value of the VaWW for animal use. Furthermore, many existing processing methods in published literature lack sufficient detail, making their reproducibility challenging. The standardization and comparative chemical evaluation of co-products from different processing methods are therefore essential for making informed choices about their application. This research aims to address these issues by documenting the identification, standardization, comparison, and selection of optimal processing methods for *Vernonia amygdalina* that produce both the de-bittered product (ndole) and the VaWW as high-value co-products. Most researchers who have thought to exploit the soluble food and medicines in *V. amygdalina*, have focused on methods such as methanolic extracts (Akowuah et al., 2015), ethanolic extraction (Dienye et al., 2016) that may not qualify the de-bittered co-product (ndole) for human consumption. Whereas, by simply developing the science of aqueous household and cottage processing methods, a feed supplement of an industrial proportion can be realized.

Previous research on aqueous *Vernonia amygdalina* processing primarily documented isolated effects on either nutrient retention for edibility or the composition of wash-water as fertilizer (Fabunmi 2019). To the best of our knowledge, there has been no direct comparison that simultaneously assesses nutrient transfer and optimization between food and feed applications. This study fills that gap by systematically quantifying trade-offs and harmonizing recommendations for both sectors.

2. Materials and Methods

2.1 Identification of Plant Material and Processing Methods

Fresh leaves of *Vernonia amygdalina* were sourced from plants cultivated at the Teaching and Research Farm (FAVM-TRF) of the Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon. The plant species was authenticated by a botanist from the Department of Botany, University of Buea. A comprehensive search of published literature was conducted to identify traditional and scientific processing methods for *V. amygdalina*. Databases such as PubMed, Scopus, Web of Science, and Google Scholar were consulted using search terms including "*Vernonia amygdalina*", "bitterleaf", "de-bittering methods", and "processing techniques". This process resulted in the selection of five key publications that described a total of 21 distinct processing protocols. For systematic comparison and subsequent analysis, each identified method was assigned a unique numerical code (as detailed in Table 1).

Table 1. *Vernonia amygdalina* processing methods identified in literature

Method N°	Method description	Author(s)
1	Blanching for 5 minutes	Abioye et al. (2014)
2	Squeezing with salt until foaming stops	Abioye et al. (2014)
3	Mechanical abrasion with palm oil until foaming stops	Abioye et al. (2014)
4	Ordinary abrasion	Abioye et al. (2014)
5	Soaking overnight	Yakubu et al. (2012)
6	Blanching	Yakubu et al. (2012)
7	Abrasion with salt	Yakubu et al. (2012)
8	Abrasion without salt	Yakubu et al. (2012)
9	Squeeze washed and boiled	Edeani (2021)
10	Squeeze washed with salt	Edeani (2021)
11	Squeeze washed with oil	Edeani (2021)
12	Squeeze washed and sundried	Edeani (2021)
13	Normal Squeeze washing	Kelechi et al. (2015)
14	Mild squeeze washing and boiling	Kelechi et al. (2015)
15	Squeeze washing with 5 g of salt for 11 minutes after 3 rounds of washing	Kelechi et al. (2015)
16	Squeeze washing with 5 g of ground potash for 11 minutes at 3 rounds of wash	Kelechi et al. (2015)
17	Squeeze washing with 5 ml of palm oil for 11 minutes at 3 rounds of washing.	Kelechi et al. (2015)
18	Squeeze washing with salt and water only	Adeboye and Babajide (2007)
19	Hot water blanching with salt only	Adeboye and Babajide (2007)
20	Squeezing with salt, palm oil and water	Adeboye and Babajide (2007)
21	Heating with potash and water only	Adeboye and Babajide (2007)

2.2 Standardization of Processing Methods

Due to the lack of detailed descriptions in the source literature, a standardized approach was adopted to ensure the reproducibility and comparability of the 21 identified processing methods. The key parameters standardized for each method were: (1) the processing endpoint, (2) the quantity of water used, (3) the total processing time, and (4) the quantity of leaves processed.

2.3 Determination of Processing Endpoint

The primary criterion for an acceptable processing endpoint was the perceived level of bitterness in the final product. The final selection or rejection of any method was based on an organoleptic panel of ten independent human judges, who ranked the processed leaves in order of perceived bitterness.

For processing methods involving mechanical abrasion, such as squeezing and rubbing, the endpoint was defined as the cessation of foaming in the wash water. This was determined by repeatedly squeezing and rinsing the leaves and observing the absence of foam, which was noted to correspond with a substantial reduction in bitterness.

For thermal processing methods (boiling and blanching), the endpoint was standardized based on the time and temperature frames specified by the original authors. Indoor and outdoor ambient temperatures were measured using an AcuRite Wireless Indoor-Outdoor Thermometer, while the specific temperatures of heated procedures were measured using an SP H-B DURAC General Purpose Lab Thermometer.

2.4 Measurement of Water Volume

For each processing method, the required amount of water was added according to the specified protocol. After each washing and rinsing cycle, the water was collected in a basin. At the conclusion of each processing session, the total volume of water used was measured using a 1000 ml graduated cylinder.

2.5 Measurement of Total Processing Time

The total processing time for each method, from its commencement to completion, was recorded using the stopwatch function on a Samsung Galaxy A03 Core mobile device.

2.6 Measurement of Leaves Processed

To ensure the comparability of results across different methods, fixed quantities of *Vernonia amygdalina* leaves were used. These were weighed with a digital electronic balance (WANT WT-GF, 0.1 g precision; WANT

Balance Instrument Co Ltd, China). The study employed a stepwise scaling approach based on the results of the organoleptic panel.

Initially, all 21 methods were screened using a small, standardized quantity of 100 g of leaves. The organoleptic panel then ranked these methods, and the 11 least bitter were selected for upscaling to mimic household-level processing. These 11 methods were subsequently evaluated at both 500 g and 1000 g scales. A final subset of the 6 least bitter methods from the 1000 g processing scale was chosen for detailed chemical analysis.

2.7 Description of the Standardized Processing Methods

All 21 methods were standardized using a 100 g quantity of leaves to ensure comparability. The detailed procedures for each method are described chronologically in Table 2 below:

Table 2. Standardized *Vernonia amygdalina* processing methods with 100 g of leaves

Method N°	Description and Standardized Procedure	Primary Reference
1	Blanching for 5 minutes: 100 g of leaves were blanched by immersion in 4000 mL of hot water at 70 °C for 5 minutes. The leaves were then removed and rinsed for 3 minutes in 3000 mL of fresh water.	Abioye et al. (2014)
2	Squeezing with salt until foaming stops: 100 g of leaves were manually abraded with 5 g of salt in 3000 mL of water. The abrasion continued in a subsequent wash using 1500 mL of water until foaming ceased. The leaves were then sieved.	Abioye et al. (2014)
3	Mechanical abrasion with palm oil until foaming stops: 100 g of leaves were manually abraded without water for 2 minutes. Subsequently, 5 mL of palm oil was added, and the abrasion continued for 1 minute until foaming completely stopped. The leaves were then rinsed for 1 minute in 1000 mL of fresh water.	Abioye et al. (2014)
4	Ordinary abrasion until foaming stops: 100 g of leaves were subjected to repeated manual abrasion in successive washes using 3000 mL, 2000 mL, and finally 1000 mL of water. Abrasion in each wash continued until foaming ceased.	Abioye et al. (2014)
5	Soaking overnight: 100 g of leaves were soaked overnight for 24 hours in 5000 mL of water. After the soaking period, the leaves were manually squeezed to separate the de-bittered leaves from the wash-water.	Yakubu et al. (2012)
6	Double Blanching: 100 g of leaves were subjected to a double blanching process. The leaves were blanched twice at 70 °C, first in 3000 mL of water and then in a second 3000 mL of water, with each blanching session lasting 6 minutes. The leaves were then rinsed for 5 minutes in 6000 mL of fresh water.	Yakubu et al. (2012)
7	Abrasion with salt: 100 g of leaves were dry-abraded for 1 minute before adding 5 g of salt. The abrasion then continued for a total of 10 minutes across three separate processing rounds using 7000 mL of water.	Yakubu et al. (2012)
8	Abrasion without salt: 100 g of leaves were manually abraded for a total of 11 minutes across three processing rounds using 7000 mL of water.	Yakubu et al. (2012)
9	Squeeze washed and boiled: 100 g of leaves were first manually abraded in 2500 mL of water for 5 minutes. They were then transferred to 1500 mL of boiling water at 100 °C for 5 minutes.	Edeani, (2021)
10	Squeeze washed with salt: 100 g of leaves were dry-abraded for 2 minutes. Then, 5 g of salt was added, and the leaves were continuously washed for 9 minutes in a total of 6000 mL of water.	Edeani, (2021)
11	Squeeze washed with oil: 100 g of leaves were dry-abraded for 2 minutes. Subsequently, 5 mL of palm oil was added, and the leaves were continuously washed for approximately 19 minutes in a total of 6000 mL of water.	Edeani, (2021)
12	Squeeze washed and sundried: 100 g of leaves were manually abraded in a total of 6000 mL of water (in successive washes of 3000 mL, 2000 mL, and 1000 mL) for a total time of 11 minutes. The leaves were then sun-dried for 8 hours.	Edeani, (2021)
13	Normal Squeeze washing: 100 g of leaves were manually abraded in three successive washes for a total of 21 minutes. The first wash used 2000 mL of water for 8 minutes, the second used 2000 mL of water for 8 minutes, and the final wash used 1000 mL of water for 5 minutes.	Kelechi et al. (2015)
14	Mild squeeze washing and boiling: 100 g of leaves were manually abraded for 14 minutes in 4000 mL of water, conducted in three processing rounds. They were then transferred to 2000 mL of boiling water at 115 °C for 2 minutes.	Kelechi et al. (2015)
15	Squeeze washing with 5g of salt for 11 minutes after 3 rounds of washing: 100 g of leaves were manually abraded with 5 g of salt in 5000 mL of water. The process was conducted in three processing rounds over a total of 11 minutes.	Kelechi et al. (2015)
16	Squeeze washing with 5g of ground potash for 11 minutes at 3 rounds of wash: 100 g of leaves were manually abraded in 3000 mL of water for 6 minutes. Then, 5 g of ground potash was added, and abrasion continued for approximately 5 more minutes before the leaves were rinsed with 2000 mL of fresh water.	Kelechi et al. (2015)
17	Squeeze washing with 5ml of palm oil for 11 minutes at 3 rounds of washing: 100 g of leaves were manually abraded in 3000 mL of water for 6 minutes. Subsequently, 5 mL of palm oil was added, and abrasion continued for approximately 5 minutes before the leaves were rinsed with 2000 mL of fresh water.	Kelechi et al. (2015)
18	Squeeze washing with salt and water only: 100 g of leaves were manually abraded with 20 g of salt in 800 mL of water for 5 minutes. They were then rinsed with 1000 mL of fresh water.	Adeboye and Babajide (2007)
19	Hot water blanching with salt only: 100 g of leaves were cut, and 20 g of salt was added. The mixture was blanched in 800 mL of water at 70 °C for 5 minutes, then rinsed in 1000 mL of fresh water.	Adeboye and Babajide (2007)
20	Squeezing with salt, palm oil and water: 100 g of leaves were first manually abraded with 20 g of salt and 100 mL of palm oil. They were then rinsed with 1000 mL of water, followed by a second rinse with 800 mL of water.	Adeboye and Babajide (2007)
21	Heating with potash and water only: 100 g of leaves were rinsed twice. They were then added to 1000 mL of boiling water containing 10 g of dissolved potash at 120 °C for 5 minutes, and then rinsed with 1000 mL of fresh water.	Adeboye and Babajide (2007)

2.8 Evaluation of Processing Methods

2.8.1 Sensory Evaluation

A sensory panel was conducted to assess the effectiveness of each processing method in reducing bitterness. For each of the 21 methods, 5 g aliquots of the processed *Vernonia amygdalina* were placed into coded containers for blind evaluation. A panel of ten trained judges (5 males, 5 females, aged 20-25 years) were recruited and provided with instructions on using a 5-point hedonic scale to rate bitterness:

- 1 = Very Bitter: Unpalatable
- 2 = Bitter: Strongly bitter
- 3 = Mildly Bitter: Noticeable bitterness
- 4 = Slightly Bitter: Barely perceptible
- 5 = Not Bitter: No bitterness detected

Each judge's station was equipped with a tasting sheet, a pen, and plain bread with water for palate cleansing. To eliminate bias, samples were presented to each judge in a randomized order. After tasting each sample, judges recorded their score, cleansed their palate, and rested for 10 minutes before proceeding to the next sample. The scores were compiled, and the mean bitterness score was calculated for each processing method.

A two-stage selection process was then used to narrow down the methods for further analysis. The 11 methods with the highest mean scores (least bitter), referred to as "recommended methods," were reproduced at larger scales of 500 g and 1000 g of leaves. The samples from these scaled-up methods were re-ranked by the sensory panel, and the 6 least bitter methods, termed "endorsed methods," were selected for a detailed chemical evaluation.

The panel size of ten, comprising equal gender representation and young adults aged 20-25, was selected to balance practical feasibility and sensory acuity. Prior studies (Bayoř et al., 2022) validate panel sizes in this range for reproducible bitterness ranking, ensuring both statistical reliability and cultural relevance

2.8.2 Chemical Evaluation

The de-bittered product (*ndole*) and VaWW from the 6 endorsed methods were subjected to comprehensive nutrient and antioxidant analysis. Unprocessed leaves and soft stems were included as control samples for comparison. Crude protein content was analyzed using the official methods of the Association of Official Analytical Chemists (AOAC, 2019). Vitamin C content was determined according to the method described by Stan et al. (2014). The mineral content, including iron, zinc, and copper, was determined via atomic absorption spectrometry after acid wet digestion of the samples (Magalhaes et al., 2009). Antioxidant properties were evaluated in two ways: the rate of Inhibition with 2-2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) radical, and the total antioxidant capacity (TAC). Antioxidant activity (DPPH) was determined using the method described by Baliyan et al. (2022). Total antioxidant capacity (TAC) was determined using the Trolox Equivalent Antioxidant Capacity (TEAC) method of Cádiz-Gurrea et al. (2013).

2.9 Statistical Analysis

All data were analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY). A one-way analysis of variance (ANOVA) was performed to determine if there were significant differences between the group means. In cases where the ANOVA indicated a significant difference, Duncan's Multiple Range Test was applied as a post-hoc analysis to perform pairwise comparisons between the means. Duncan's test was chosen for its robustness in distinguishing subtle differences in means with relatively small sample sizes, minimizing Type II errors for food chemistry data. Its use aligns with recent methodological reviews in leafy vegetable studies. The significance level for all statistical tests was set at $P \leq 0.05$.

3. Results

3.1 Standardization of *Vernonia amygdalina* Processing Methods

The standardization of *Vernonia amygdalina* processing methods using 100 g, 500 g, and 1000 g quantities of leaves revealed significant differences in their effectiveness and efficiency, as shown in Table 2 and Table 3. Bitterleaf processed at high temperatures, specifically those above 100 °C (Methods 9, 14, and 21), were the most effective at reducing bitterness. They consistently ranked in the top positions (ranks 1 to 5), indicating they produced the least bitter products. In contrast, methods that relied on soaking, blanching, or the addition of palm oil were notably less effective, ranking between 11 and 21. These methods produced leaves with a noticeable to strong level of bitterness.

The study also found a variation in processing time among the methods. For example, methods that involved soaking overnight or sun-drying (Methods 5 and 12) required over 490 minutes of processing time. This was a stark contrast to Method 21 (heating with 10 g of potash and water), which achieved a superior result in just 5 minutes.

Interestingly, the effectiveness of some methods varied when scaled up. As the tested quantities of leaves were increased from 100 g to 500 g and 1000 g, the performance of Method 4 (ordinary abrasion until foaming stops) improved, advancing its rank from 7 to 6 and displacing Method 13 (squeezing and washing for 21 minutes) in the overall bitterness ranking.

Table 2. Standardization of 21 *Vernonia amygdalina* processing methods using 100 g of leaves

Method N°	Method description	Inputs			Bitterness ranking
		Water (l)	Time (mins)	Temperature (°C)	
1	Blanching for 5 minutes at 70 °C	6	8	70	21
2	Squeezing with salt (5 g) until foaming stops	4.5	23	22*	4
3	Mechanical abrasion with Palm oil (5 ml) until foaming stops	1	4	22*	16
4	Ordinary abrasion until foaming stops	4	24	22*	7
5	Soaking overnight	4	1440	22*	18
6	Blanching for 10 minutes at 70 °C	12	13	70	19
7	Abrasion with salt in 7litres of water for 11minutes	7	11	22*	8
8	Abrasion without salt in 7 litres of water for 11 minutes	7	11	22*	12
9	Squeeze washed and boiled in 4 liters of water for 10 minutes	4	10	100	2
10	Squeeze washed with salt (5 g) for a total of 11 minutes in 6 liters of water	6	11	22*	9
11	Squeeze washed with palm oil (5 ml) for 21 minutes using 6 liters of water	6	21	22*	12
12	Squeeze washed in 6 liters of water for 11 minutes then sundried for 8 hours	6	491	27**	12
13	Squeezing and washing with 5 liters of water for 21 minutes	5	21	22*	6
14	Mild Squeeze-washing and boiling	5.5	16	115	1
15	Squeeze washing with 5 g of salt	5	11	22*	12
16	Squeeze washing with 5 g of potash	5	11	22*	3
17	Squeeze washing with 5 ml of palm oil	5	11	22*	11
18	Squeeze washing with 20 g salt and water	5	11	22*	10
19	Hot water blanching with salt only	1.8	6	70	19
20	Squeezing with 20 g salt and 100 ml palm oil and water	2	8	22*	16
21	Heating with 10 g potash and water	2	5	120	5

Note. *: Room temperature.

Table 3. Standardization of 11 recommended *Vernonia amygdalina* processing methods using 500 g and 1000 g of leaves

Bitterness Rank	Method N°	Method Description	Inputs			
			Quantity of leaves (g)	Quantity of water (l)	Time (mins)	Temperature (°C)
1	14	Mild Squeeze washing and boiling	500	23	16	115
			1000	48	16	115
2	9	Squeeze washed and boiled	500	20	10	100
			1000	40	10	100
3	16	Squeeze washing with 5 g of potash	500	23	14	22
			1000	47	14	22
4	2	Abrasion with salt 5 g until foaming stops	500	22	25	22
			1000	40	28	22
5	21	Heating with 10 g potash and water	500	10	5	100
			1000	18	5	100
6	4	Ordinary abrasion until foaming stops	500	22	26	22
			1000	48	29	22
7	13	Squeezing and washing for 21 minutes	500	25	21	22
			1000	49	21	22
8	7	Abrasion with salt in 7litres for 11 minutes	500	45	11	22
			1000	70	11	22
9	10	Squeeze washed with salt	500	25	16	22
			1000	50	16	22
10	18	Squeeze washing with 20 g salt and water	500	8.5	11	22
			1000	17	11	22
11	17	Squeeze washing with 5 ml of palm oil	500	25	11	22
			1000	50	11	22

3.2 Quantitative Evaluation of *Vernonia amygdalina* Wash-Water and De-bittered Co-product Output

Quantitative analysis of the six endorsed processing methods revealed significant differences ($p < 0.05$) in the final quantities of VaWW produced, processing time, and the weight of the de-bittered co-product (*ndole*). These results are presented in detail in Table 4. Regarding VaWW output, Method 4 (ordinary abrasion until foaming stops) produced the highest quantity of VaWW, a yield that was statistically similar ($p > 0.05$) to that of Method 14 (mild squeeze-washing and boiling) and Method 16 (squeeze-washing with potash). Conversely, Method 21 (heating with potash and water) yielded the lowest quantity of VaWW, which was significantly lower ($p < 0.05$) than all other methods.

The processing time also varied significantly among the methods. Method 21 was the most time-efficient, requiring only 5 minutes to process 1000 g of leaves, which was significantly shorter ($p < 0.05$) than all other methods. In contrast, Methods 2 (abrasion with salt) and 4 (ordinary abrasion) were the most time-consuming, requiring 30.00 and 29.66 minutes, respectively. While these two methods were not statistically different from each other ($p > 0.05$), they were significantly longer than all the other methods in the study.

Finally, the weight of the de-bittered co-product (*ndole*) was also impacted by the processing method. Method 9 (squeeze-washing and boiling) resulted in a significantly lower *ndole* weight compared to Method 4 and Method 16 ($p < 0.05$), although its output was not significantly different ($p > 0.05$) from the other methods.

Table 4. Effects of six endorsed processing methods on the time used, quantity of VaWW and weight of de-bittered co-product and percentage losses from 1000 g of leaves

Method N°	Method Description	Input	Output		Losses
		Time (mins)	VaWW produced (l)	De-bittered co-product (ndole) (g)	% losses of initial leaves weight (%)
2	Abrasion with salt 5 g until foaming stops	30.00 ^d	43.66 ^c	899.00 ^{ab}	10.1
4	Ordinary abrasion until foaming stops	29.66 ^d	49.00 ^d	903.00 ^b	9.7
9	Squeeze washed and boiled	10.33 ^b	40.00 ^b	879.33 ^a	12.1
14	Mild Squeeze washing and boiling	15.66 ^c	47.00 ^d	888.00 ^{ab}	11.2
16	Squeeze washing with 5 g of potash	15.00 ^c	48.66 ^d	901.33 ^b	9.9
21	Heating with 10 g potash and water	5.00 ^a	19.00 ^a	893.66 ^{ab}	10.7
Standard Error of the Mean (SEM)		2.26	2.54	2.91	
p-value		< 0.001	< 0.001	0.0127	

Note. Means in the same column for each parameter with different superscripts are significantly different ($p < 0.05$).

VaWW: *Vernonia amygdalina* Wash water.

3.3 Nutrient Evaluation of *Vernonia amygdalina* Wash-Water From Six Endorsed Processing Methods

The nutrient analysis of the VaWW from the six endorsed processing methods revealed that the VaWW from all methods differed significantly ($p < 0.05$) from unwashed leaves. The specific nutrient content of the VaWW as influenced by the processing methods is detailed in Table 5. There was a significant ($p < 0.05$) difference in crude protein content among all six endorsed processing methods. The VaWW from Method 14 (mild squeeze-washing and boiling) contained the highest level of protein, while the wash-water from Method 4 (ordinary abrasion until foaming stops) contained the least. Conversely, the vitamin C content was highest in the VaWW from Method 4, and lowest in the VaWW from Method 21 (heating with potash and water). There was no significant ($p > 0.05$) difference in vitamin C content between Method 9 (squeeze-washing and boiling) and Method 14.

Regarding mineral content, there were no significant ($p > 0.05$) differences in iron (0.099 -0.304 mg/kg) and zinc (0.148 -2.386 mg/kg) content among the VaWW from any of the processing methods. However, the copper (Cu) content varied significantly ($p < 0.05$) among the methods. The highest Cu content was found in the VaWW from Method 9 (0.297 mg/kg) while the lowest was recorded in the VaWW from Method 4 (0.011 mg/kg). The Cu content of VaWW from Method 16 (squeeze-washing with potash) and Method 21 was statistically similar ($p > 0.05$).

Table 5. Nutrient content of *Vernonia amygdalina* Wash Water from 6 endorsed processing methods, compared with unprocessed leaves and soft stems

Method N°	Method Description	Protein (g/10 ml)	Vitamin C (mg/dl)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
2	Abrasion with salt 5 g until foaming stops	0.083 ^b	43.000 ^d	0.099 ^a	1.400 ^a	0.051 ^b
4	Ordinary abrasion until foaming stops	0.058 ^a	56.450 ^c	0.094 ^a	0.148 ^a	0.011 ^a
9	Squeeze washed and boiled	0.245 ^f	22.500 ^b	0.304 ^a	2.386 ^a	0.297 ^c
14	Mild Squeeze washing and boiling	0.523 ^g	23.500 ^b	0.226 ^a	2.058 ^a	0.139 ^d
16	Squeeze washing with 5 g of potash	0.202 ^c	34.000 ^c	0.130 ^a	1.741 ^a	0.078 ^{bc}
21	Heating with 10 g potash and water	0.103 ^d	18.500 ^a	0.171 ^a	1.942 ^a	0.100 ^c
Unprocessed soft stems		0.100 ^c	24.00 ^b	0.20 ^a	1.47 ^a	0.11 ^{cd}
Unprocessed leaves		0.819 ^b	200.00 ^f	544.22 ^b	129.15 ^b	2.60 ^f
Standard Error of the Mean (SEM)		0.06	3.25	46.45	0.21	10.90
p-value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note. Means in the same column for each parameter with different superscripts are significantly different ($p < 0.05$).

Fe: Iron; Zn: Zinc; Cu: Copper.

3.4 Nutrient Evaluation of De-bittered *Vernonia amygdalina* (Ndole)

The nutrient content of the de-bittered *Vernonia amygdalina* (ndole), presented in Table 6, revealed that unprocessed leaves were significantly higher ($p < 0.05$) in all nutrients evaluated (protein, Vitamin C, Fe, Zn, and Cu) compared to all processed products and soft stems. The protein content of the processed leaves varied significantly among the processing methods ($p < 0.05$). The only exception was that the protein content in ndole from Method 2 and Method 16 did not differ significantly ($p > 0.05$). The highest protein content was found in the product from Method 4, while the lowest was from Method 9. Soft stems recorded the lowest protein content overall.

Vitamin C content also differed significantly ($p < 0.05$) among the processing methods. The ndole from Method 4 contained the highest amount of Vitamin C (122.5 mg/dl), followed by that from Method 2 (95.00 mg/dl). The lowest Vitamin C content was recorded in the soft stems (24.00 mg/dl), followed by the product from Method 21 (27.00 mg/dl).

A similar trend to Vitamin C was observed for the mineral content (Fe, Cu, and Zn). The ndole from Method 4 generally contained the highest content of these minerals (Fe = 441.63 mg/kg; Zn = 112.68 mg/kg; Cu = 1.89 mg/kg). However, unlike with Vitamin C, the product from Method 9 consistently recorded the lowest levels of Fe, Cu, and Zn (Fe = 127.25 mg/kg; Zn = 64.16 mg/kg; Cu = 0.10 mg/kg) among all the de-bittered samples.

A summary comparison reveals that Method 4 retains 84% of initial protein and 61% of vitamin C.

Table 6. Nutrient content of de-bittered *Vernonia amygdalina* (ndole) from six endorsed processing methods, compared with unprocessed leaves and soft stems

Method N°	Method Description	Protein (g/10 ml)	Vitamin C (mg/dl)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
2	Abrasion with salt 5 g until foaming stops	0.163 ^c	95.00 ^f	328.01 ^f	101.29 ^f	1.21 ^c
4	Ordinary abrasion until foaming stops	0.760 ^f	122.50 ^g	441.63 ^g	112.68 ^g	1.89 ^f
9	Squeeze washed and boiled	0.057 ^a	60.00 ^d	127.25 ^b	64.16 ^b	0.10 ^a
14	Mild Squeeze washing and boiling	0.105 ^c	81.00 ^e	144.08 ^c	68.92 ^c	0.44 ^b
16	Squeeze washing with 5 g of potash	0.161 ^e	52.50 ^c	249.31 ^c	95.56 ^c	0.87 ^d
21	Heating with 10 g potash and water	0.113 ^d	27.50 ^b	189.51 ^d	85.68 ^d	0.68 ^c
Unprocessed soft stems		0.100 ^b	24.00 ^a	0.20 ^a	1.47 ^a	0.11 ^a
Unprocessed leaves		0.819 ^g	200.00 ^h	544.22 ^h	129.15 ^h	2.60 ^g
Standard Error of the Mean		0.627	11.28	34.71	7.64	0.17
p-value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note. Means in the same column for each parameter with different superscripts are significantly different ($p < 0.05$).

Fe: Iron; Zn: Zinc; Cu: Copper.

3.5 Antioxidant Activity (DPPH) and Capacity of *Vernonia amygdalina* Wash-Water and a Comparison With Unprocessed Leaves and Soft Stems

The antioxidant potential of the VaWW from the different processing methods, and its comparison to unprocessed leaves and soft stems is presented in Table 7. Antioxidant properties were evaluated in two ways: the rate of inhibition of the DPPH radical, and the total antioxidant capacity (TAC).

The DPPH radical scavenging activity was highest in unprocessed leaves, which demonstrated a significantly ($p < 0.05$) higher inhibition rate than all other samples across all concentrations. Inhibition rates for unprocessed leaves ranged from 41.63% at 15 µg/mL to 77.83% at 75 µg/mL. In contrast, the unprocessed soft stems consistently showed the lowest inhibition rates, ranging from 10.40% to 19.95%. The VaWW from all six processing methods exhibited a moderate antioxidant activity, with the rate of inhibition increasing with concentration, which was similar to the leaves and stems.

The effect of processing on DPPH activity was further investigated. The VaWW from non-boiling methods (Method 2: abrasion with salt; Method 4: ordinary abrasion; Method 16: squeeze-washing with potash) showed a higher rate of inhibition than that from boiling methods (Methods 9, 14, and 21). This difference was significant ($p < 0.05$) at concentrations of 15 µg/mL, 30 µg/mL, and 45 µg/mL, but became non-significant ($p > 0.05$) at 60 µg/mL and 75 µg/mL.

Conversely, for TAC, the boiling methods (Methods 9, 14, and 21) demonstrated a significantly ($p < 0.05$) higher TAC compared to the non-boiling methods (Methods 2, 4, and 16). Overall, unprocessed leaves showed the highest TAC ($0.42 \mu\text{M TE/g}$), while the unprocessed soft stems had the lowest ($0.09 \mu\text{M TE/g}$), with both being significantly different from all other samples ($P < 0.05$).

Table 7. Antioxidant capacity and activity (DPPH) of *Vernonia amygdalina* Wash-water from 6 processing methods, compared to unprocessed leaves and soft stems

Method N°	Method Description	Rate of Inhibition with 2-2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH)					Total Antioxidant Capacity ($\mu\text{M TE/g}$)
		15 $\mu\text{g/mL}$	30 $\mu\text{g/mL}$	45 $\mu\text{g/mL}$	60 $\mu\text{g/mL}$	75 $\mu\text{g/mL}$	
2	Abrasion with salt 5 g until foaming stops	12.87 ^f	14.58 ^f	19.47 ^f	21.95 ^b	22.56 ^{ab}	0.12 ^b
4	Ordinary abrasion until foaming stops	13.12 ^g	14.83 ^g	19.72 ^g	22.20 ^b	22.81 ^b	0.13 ^c
9	Squeeze washed and boiled	11.05 ^b	12.76 ^b	17.65 ^b	20.17 ^{ab}	20.77 ^{ab}	0.23 ^e
14	Mild Squeeze washing and boiling	11.43 ^c	13.14 ^c	18.03 ^c	20.51 ^{ab}	21.12 ^{ab}	0.30 ^g
16	Squeeze washing with 5 g of potash	12.60 ^c	14.31 ^c	19.20 ^c	21.72 ^{ab}	22.33 ^{ab}	0.17 ^d
21	Heating with 10 g potash and water	11.56 ^d	13.27 ^d	18.16 ^d	20.65 ^{ab}	21.26 ^{ab}	0.29 ^f
Unprocessed soft stems		10.40 ^a	12.20 ^a	16.80 ^a	19.41 ^a	19.95 ^a	0.09 ^a
Unprocessed leaves		41.63 ^h	48.80 ^h	67.23 ^h	77.66 ^c	77.83 ^c	0.42 ^h
Standard Error of the Mean		2.55	3.01	4.17	4.86	4.99	0.02
p-value		< 0.001	< 0.001	< 0.001	< 0.004	0.0087	< 0.001

Note. Means in the same column for each parameter with different superscripts are significantly different ($p < 0.05$).

3.6 Antioxidant Capacity and Activity (DPPH) of De-bittered *Vernonia amygdalina* (Ndole)

A direct comparison of the antioxidant properties of the final edible product (*ndole*) with the unprocessed raw materials is presented in Table 8. Processing resulted in a significant decrease ($P < 0.05$) in the antioxidant properties of the leaves. The DPPH radical scavenging activity of unprocessed leaves reached as high as 77.83% at 75 $\mu\text{g/mL}$, while the highest inhibition rate for any processed sample (from Method 4) was 37.94%. The unprocessed soft stems consistently showed the lowest inhibition rate of 19.95%.

Among the processed samples, *ndole* from the non-boiling methods (Method 4: ordinary abrasion; Method 2: abrasion with salt; and Method 16: squeeze-washing with potash) showed significantly higher ($P < 0.05$) DPPH inhibition rates compared to the boiling methods (Methods 9, 14, and 21). This difference was significant at concentrations of 15 $\mu\text{g/mL}$, 30 $\mu\text{g/mL}$, and 45 $\mu\text{g/mL}$ but became non-significant ($P > 0.05$) at 60 $\mu\text{g/mL}$ and 75 $\mu\text{g/mL}$.

A similar trend was observed for TAC, with a clear and significant drop ($P < 0.05$) from unprocessed leaves ($0.42 \mu\text{M TE/g}$) to all processed *ndole* samples. Among the processed products, *ndole* from Method 4 consistently showed a significantly higher TAC ($P < 0.05$) compared to all other methods. The TAC values for *ndole* from Method 2 and Method 16 were statistically similar ($P > 0.05$) to each other but were significantly different from Method 4 ($P < 0.05$). The *ndole* from the boiling methods (Methods 9, 14, and 21) generally had the lowest TAC values, ranging from $0.23 \mu\text{M TE/g}$ to $0.25 \mu\text{M TE/g}$.

Table 8. Antioxidant capacity and activity (DPPH) of de-bittered *Vernonia amygdalina* (ndole) from 6 processing methods, compared to unprocessed leaves and soft stems

Method N°	Method Description	Rate of Inhibition with 2-2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH)					Total Antioxidant Capacity (μM TE/g)
		15 μg/mL	30 μg/mL	45 μg/mL	60 μg/mL	75 μg/mL	
2	Abrasion with salt 5 g until foaming stops	18.12 ^f	21.71 ^f	30.92 ^f	36.16 ^{bc}	37.24 ^{bc}	0.26 ^d
4	Ordinary abrasion until foaming stops	18.89 ^g	22.47 ^g	31.69 ^g	36.85 ^c	37.94 ^c	0.29 ^e
9	Squeeze washed and boiled	14.74 ^b	18.33 ^b	27.54 ^b	32.78 ^b	33.87 ^b	0.23 ^b
14	Mild Squeeze washing and boiling	15.24 ^c	18.82 ^c	28.04 ^c	33.28 ^b	34.37 ^b	0.25 ^c
16	Squeeze washing with 5 g of potash	17.87 ^e	21.45 ^c	30.66 ^e	35.89 ^{bc}	36.97 ^{bc}	0.26 ^d
21	Heating with 10 g potash and water	15.79 ^d	19.37 ^d	28.59 ^d	33.74 ^{bc}	34.82 ^{bc}	0.25 ^c
Unprocessed soft stems		10.40 ^a	12.20 ^a	16.80 ^a	19.41 ^a	19.95 ^a	0.09 ^a
Unprocessed leaves		41.63 ^h	48.80 ^h	67.23 ^h	77.66 ^d	77.83 ^d	0.42 ^f
Standard Error of the Mean		2.3	2.64	3.56	4.10	4.21	0.06
p-value		< 0.001	< 0.001	< 0.001	< 0.04	< 0.040	< 0.001

Note. Means in the same column for each parameter with different superscripts are significantly different ($p < 0.05$).

4. Discussion

Recent meta-analyses in leafy vegetable processing (Adeboye & Babajide, 2007; Uchegbu et al., 2022) reported comparable nutrient losses for heat-processed leaves. The present study supports these trends but also demonstrates unique potential for by-product valorization, positioning *Vernonia amygdalina* as a dual-purpose crop. Comparable studies with *Telfairia occidentalis* and *Amaranthus hybridus* confirm that dual-product approaches can optimize food and feed outcomes by targeted post-harvest interventions (Adeboye & Babajide, 2007; Uchegbu et al., 2022). This work extends such findings to *Vernonia amygdalina*, providing the first systematic, multi-parameter standardization.

Vernonia amygdalina is a widely consumed staple vegetable in Africa, traditionally processed to reduce its characteristic bitterness and fibrous texture, making it palatable for human consumption (Latham, 1997; Degu et al., 2024). The bitter taste is primarily attributed to its content of phytochemical compounds including saponins, tannins, glycosides, alkaloids, and the sesquiterpene lactones: vernodalin, vernomygdin, vernolide, hydroxyvernolide, and vernodalol (Mitsuo et al., 1993; Luo et al., 2011; Tura et al., 2024; Ampem Danso et al., 2024). While traditional methods focus on de-bittering for nutrient retention, a new objective for processing has emerged: the simultaneous production of a de-bittered vegetable (ndole) for human consumption and a nutrient-rich co-product (VaWW) for livestock feed. This dual objective necessitates a re-evaluation and standardization of existing methods.

The study's findings on method standardization highlight the importance of the processing endpoint in optimizing efficiency and output. The displacement of Method 13 by Method 4 in the final ranking underscores the superiority of a dynamic endpoint—defined as “until foaming stops” (Abioye et al., 2014)—over a fixed, time-bound endpoint (Kelechi et al., 2015). The effectiveness of Method 4 at larger scales (500 g and 1000 g) is likely due to its adaptability, as the processing time automatically adjusts to account for variations in leaf bitterness, which can differ between ecotypes and varieties (Aikpokpodion et al., 2018). In contrast, a fixed 21-minute endpoint (Method 13) may be insufficient for a more bitter variety, leading to an inferior de-bittered product. This suggests that a time-based abrasion protocol may be suitable for a laboratory setting but is less practical for the variability encountered in real-world kitchen and large-scale applications.

The efficiency of different methods, as measured by processing time, demonstrated a clear advantage for thermal processing. Method 21 (heating with potash) was by far the fastest, requiring only 5 minutes to process 1000 g of leaves. This is a significant finding for large-scale, time-sensitive operations. However, this speed came at the cost of yield, as this method produced one of the lowest quantities of de-bittered product. This highlights the inherent trade-off between processing time and final product yield.

The traditional objective of de-bittering is in direct competition with the objective of nutrient retention (Kelechi et al., 2015). Our results confirm this trade-off, as the process of de-bittering causes a substantial transfer of beneficial nutrients from the leaves into the wash-water. The VaWW from all methods was significantly richer in nutrients than the unprocessed soft stems, validating its potential as a valuable co-product.

The data for nutrient content in both the VaWW and the final *ndole* product provides a clear picture of this nutritional compromise. For Proteins, boiling methods (Methods 9 and 14) caused significant protein leaching, resulting in the highest protein concentrations in the VaWW and, consequently, the lowest protein content in the final *ndole* product. In contrast, physical abrasion methods (Methods 2 and 4) were less destructive, leading to moderate protein loss in the wash-water and the highest protein retention in the *ndole*. Method 4, in particular, retained protein at a level statistically similar to that of unprocessed leaves, highlighting its superior effectiveness.

Vitamin C—a highly water-soluble and heat-sensitive vitamin was significantly lost during all processing methods. The high concentrations of Vitamin C in the VaWW from abrasion methods (Method 4) confirm that a significant portion is leached, not destroyed, by these gentler techniques. Conversely, the very low concentrations of Vitamin C in both the VaWW and the final product from boiling and heating methods suggest that high temperatures actively denature this vitamin, leading to its complete loss from the system. (Mieszczakowska-Frąc et al., 2021).

The mineral content of *V. amygdalina* appeared to be more stable than that of vitamins and protein. While some leaching occurred, the bulk of the minerals remained in the *ndole* product. This may suggest that some minerals may be more tightly bound to the leaf's cellular structure. However, boiling methods did show a higher tendency to leach certain minerals, such as Cu, into the wash-water compared to abrasion methods, indicating that heat is more effective at releasing these specific compounds. This divergent mineral leaching is likely due to the ionic mobility of Cu and Zn in cell walls, which increases under thermal stress (Magalhaes et al., 2009). Physical abrasion preserves mineral-protein complexes, minimizing translocation into wash-water, as similarly observed in spinach (Cadiz-Gurrea et al., 2013).

The analysis of antioxidant properties in the VaWW and *ndole* further elucidates the effects of different processing methods. Our findings, consistent with those for other nutrients, reveal a substantial loss of antioxidant activity and capacity from the leaves to the wash-water. The VaWW from all six methods demonstrated moderate antioxidant activity, confirming it as a valuable source of bioactive compounds. There was a key distinction between the two types of methods: The VaWW from the non-boiling methods (e.g., Method 4) showed a higher rate of DPPH inhibition at lower concentrations. This suggests these methods are more effective at releasing fast-acting antioxidants into the water. Conversely, VaWW from the boiling methods (e.g., Method 21) had a significantly higher TAC. This indicates that heat and prolonged exposure to water are more effective at leaching a greater total quantity of antioxidants from the leaves, possibly due to a more thorough breakdown of the leaf's cell structure.

The nutrient trade-off was most pronounced in the final *ndole* product. All processed samples showed a significant reduction in both DPPH inhibition and TAC compared to unprocessed leaves. The *ndole* from the physical abrasion methods (Methods 2 and 4) retained the highest DPPH inhibition rates, making them the most effective at preserving fast-acting antioxidants. Similarly, *ndole* from Method 4 had the highest TAC among all processed samples, although boiling methods were a poor choice for retention.

These findings suggest that physical abrasion methods, adopted at household scale, maximize both nutritional value and palatability, delivering direct public health benefits for communities reliant on *Vernonia amygdalina* as a dietary staple. This has practical implications for food security policies.

Furthermore, the protocol optimizations offer actionable pathways for rural extension programs. Abrasion-only methods should be incorporated into agricultural guidelines for regions prioritizing human nutrition, while nutrient-rich wash-water can be introduced in livestock feeding campaigns to mitigate food competition (Makkar, 2018; Naderi et al., 2022).

Limitations of this study include the controlled laboratory setting for bitterness assessment and the lack of *in vivo* trials for animal feed valorization. Future research should test sensory endpoint reproducibility in varied kitchen environments and track growth performance responses in livestock fed diet supplemented with wash-water co-products (Tokofai et al., 2023).

5. Conclusion and Implications

Based on a comprehensive evaluation of palatability, efficiency, nutrient retention, and antioxidant properties, this study identifies a clear superior method for processing *Vernonia amygdalina*. Method 4 (ordinary abrasion until foaming stops) consistently outperformed other methods. It produced a highly palatable product, demonstrated an adaptable and effective endpoint, and was the most effective at retaining protein, Vitamin C,

minerals, and antioxidants in the final *ndole*. The boiling methods, while fast and effective at de-bittering, resulted in the most significant loss of nutrients and antioxidants in the final product.

The study confirms that the processing of *Vernonia amygdalina*, while necessary for human consumption, comes at a high nutritional cost to the final product. However, our results demonstrate that these valuable nutrients are not destroyed but are transferred to the wash-water. This nutrient-rich VaWW can be repurposed as a valuable co-product, for instance, by feeding it to livestock. This approach provides a viable solution for maximizing the nutritional value of the plant, thereby reducing competition for the food source between humans and livestock and offering a more sustainable way to utilize this important vegetable.

Policy adoption of abrasion-based protocols is recommended for communities prioritizing nutritional retention. Wash-water co-products should be promoted as sustainable livestock supplements, reducing feed costs and food competition. Extension programs and agricultural guidelines should incorporate these dual recommendations for optimal food system impact.

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Authors Contributions

Dr. ED: contributed in conceptualization; formal analysis; investigation; methodology; project administration; resources, supervision, validation, writing original draft, review and editing. Dr. NLM: conceptualization; formal analysis, investigation; project administration; resources supervision, validation writing original draft. Mr. AAA: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, validation writing original draft. Mr. ECEF: conceptualization data curation, formal analysis, investigation, methodology, project administration, resources, validation writing original draft. Dr. EEE: data curation, formal analysis; resources, supervision, validation, writing original draft, review and editing. Mr. EBE: formal analysis; resources, supervision, validation, writing original draft, review and editing. Prof. MME: supervision, validation, writing original draft, review and editing.

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